Unbuckling lipodystrophy from insulin resistance and hypertension

Robert A. Hegele, Todd Leff

Commentary

Lipodystrophy and insulin resistance are the core features of human PPARγ deficiency states. Metabolic complications in PPARγ deficiency, such as hypertension, have been considered to be secondary to insulin resistance. However, a new mouse model that expresses the analog of a human *PPARG* mutation displays minimal lipodystrophy and insulin resistance but rather severe hypertension. Furthermore, the mutant protein appears to directly modulate the renin-angiotensin system in adipose tissue, providing evidence of the pleiotropic effects of PPARγ.

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hope has often been more hype than real-ization. The story of Marfan syndrome and mutations in the *FBN1* gene illustrates that identifying a mutation is only the first step in understanding the molecular pathophysi-ology of the disorder and that the pathway to treatment may be both complex and full of insights into unexpected areas of biology (24, 25). Perhaps in a short time we may see the use of TGF-β blockade in preference to β-(adrenergic) blockade as a more effective treatment of many aspects of Marfan syn-drome — and treatment is the ultimate goal of understanding this complex phenotype.

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1. Dietz, H.C., et al. 1991. Marfan syndrome caused by a recurrent de novo missense mutation in the fibrillin gene. *Nature.* **352**:337–339.
2. Sakai, L.Y., Keene, D.R., and Engvall, E. 1986. Fibrillin, a new 350-kD glycoprotein, is a com-ponent of extracellular microfibrils. *J. Cell Biol.* **103**:2499–2509.
3. Hollister, D.W., Godfrey, M., Sakai, L.Y., and Pyeritz, R.E. 1990. Immunohistologic abnormalities of the microfibrillar-fiber system in the Marfan syndrome. *N. Engl. J. Med.* **323**:152–159.
4. Lee, B., et al. 1991. Linkage of Marfan syndrome and a phenotypically related disorder to two different fibrillin genes. *Nature.* **352**:330–334.
5. Magenis, R.E., Maslen, C.L., Smith, L., Allen, L., and Sakai, L.Y. 1991. Localization of the fibrillin (FBN) gene to chromosome 15, band q21.1. *Genomics.* **11**:346–351.
6. Milewicz, D.M., Pyeritz, R.E., Crawford, E.S., and Byers, P.H. 1992. Marfan syndrome: defective synthe-sis, secretion, and extracellular matrix formation of fibrillin by cultured dermal fibroblasts. *J. Clin. Invest.* **89**:79–86.
7. The FBN1 mutations database. http://www.umd.be:2030/.
8. Faivre, L., et al. 2003. In frame fibrillin-1 gene dele-tion in autosomal dominant Weill-Marchesani syn-drome. *J. Med. Genet.* **40**:34–36.
9. Ades, L.C., et al. 2002. Segregation of a novel FBN1 gene mutation, G1796E, with kyphoscoliosis and radiographic evidence of vertebral dysplasia in three generations. *Am. J. Med. Genet.* **109**:261–270.
10. Katzke, S., et al. 2002. TGGE screening of the entire FBN1 coding sequence in 126 individuals with marfan syndrome and related fibrillinopathies. *Hum. Mutat.* **20**:197–208.
11. Sood, S., Eldadah, Z.A., Krause, W.L., McIntosh, I., and Dietz, H.C. 1996. Mutation in fibrillin-1 and the Marfanoid-craniosynostosis (Shprintzen-Goldberg) syndrome. *Nat. Genet.* **12**:209–211.
12. Bona, C.A., et al. 1997. Structure of the mutant fibrillin-1 gene in the tight skin (TSK) mouse. *DNA Res.* **4**:267–271.
13. Eldadah, Z.A., Brenn, T., Furthmayr, H., and Dietz, H.C. 1995. Expression of a mutant human fibrillin allele upon a normal human or murine genetic background recapitulates a Marfan cellular phe-notype. *J. Clin. Invest.* **95**:874–880.
14. Judge, D.P., et al. 2004. Evidence for a critical con-tribution of haploinsufficiency in the complex pathogenesis of Marfan syndrome. *J. Clin. Invest.* **114**:172–181. doi:10.1172/JCI200420641.
15. Putnam, E.A., Zhang, H., Ramirez, F., and Milewicz, D.M. 1995. Fibrillin-2 (FBN2) mutations result in the Marfan-like disorder, congenital contractural arachnodactyly. *Nat. Genet.* **11**:456–458.
16. Corson, G.M., Charbonneau, N.L., Keene, D.R., and Sakai, L.Y. 2004. Differential expression of fibrillin-3 adds to microfibril variety in human and avian, but not rodent, connective tissues. *Genomics.* **83**:461–472.
17. Annes, J.P., Munger, J.S., and Rifkin, D.B. 2003. Mak-ing sense of latent TGFbeta activation. *J. Cell. Sci.* **116**:217–224.
18. Nakajima, Y., Miyazono, K., and Nakamura, H. 1999. Immunolocalization of latent transforming growth factor-beta binding protein-1 (LTBP1) dur-ing mouse development: possible roles in epithelial and mesenchymal cytodifferentiation. *Cell Tissue Res.* **295**:257–267.
19. Saharinen, J., and Keski-Oja, J. 2000. Specific sequence motif of 8-Cys repeats of TGF-beta bind-ing proteins, LTBPs, creates a hydrophobic interac-tion surface for binding of small latent TGF-beta. *Mol. Biol. Cell.* **11**:2691–2704.
20. Kaartinen, V., and Warburton, D. 2003. Fibrillin con-trols TGF-beta activation. *Nat. Genet.* **33**:331–332.
21. Saito, S., et al. 1999. Characterization of mutated protein encoded by partially duplicated fibrillin-1 gene in tight skin (TSK) mice. *Mol. Immunol.* **36**:169–176.
22. Neptune, E.R., et al. 2003. Dysregulation of TGF-beta activation contributes to pathogenesis in Marfan syndrome. *Nat. Genet.* **33**:407–411.
23. Hutchinson, S., et al. 2003. Allelic variation in normal human FBN1 expression in a family with Marfan syndrome: a potential modifier of phenotype? *Hum. Mol. Genet.* **12**:2269–2276.
24. Aoyama, T., Tynan, K., Dietz, H.C., Francke, U., and Furthmayr, H. 1993. Missense mutations impair intracellular processing of fibrillin and microfibril assembly in Marfan syndrome. *Hum. Mol. Genet.* **2**:2135–2140.
25. Frischmeyer, P.A., and Dietz, H.C. 1999. Nonsense-mediated mRNA decay in health and disease. *Hum. Mol. Genet.* **8**:1893–1900.
26. Kaartinen, V., and Warburton, D. 2003. Fibrillin con-trols TGF-β activation. *Nat. Genet.* **33**:331–332.

Unbuckling lipodystrophy from insulin resistance and hypertension

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Lipodystrophy and insulin resistance are the core features of human PPARγ deficiency states. Metabolic complications in PPARγ deficiency, such as hyper-tension, have been considered to be secondary to insulin resistance. However, a new mouse model that expresses the analog of a human *PPARG* mutation displays minimal lipodystrophy and insulin resistance but rather severe hypertension (see the related article beginning on page 240). Furthermore, the mutant protein appears to directly modulate the renin-angiotensin system in adipose tissue, providing evidence of the pleiotropic effects of PPARγ.

The commonly occurring metabolic syn-drome (MetS) is considered to result from complex gene-environment interactions and has been associated with future onset of type 2 diabetes mellitus (T2DM) (1) and both all-cause and cardiovascular mortality (2). MetS is defined clinically according to deviation from threshold values for three or more of five quantitative traits; namely, waist circumference, blood pressure, and plasma concentrations of glucose, high-density lipoprotein cholesterol, and triglyc-eride (3). Insulin resistance has long been considered the core biochemical defect linking these metabolic disturbances, which are strongly correlated among and within patients, suggesting the existence of common underlying molecular mecha-nisms. No molecule is more central to the metabolic and vascular pathways of MetS than PPARγ (4). The effect of altered activ-ity of the PPARγ receptor on whole-body insulin sensitivity has been appreciated for years. For instance, in both mice and humans, activating PPARγ ligands has ben-

Table 1

Comparison of selected phenotypes of human lipodystrophies FPLD2 and FPLD3 and the mouse $Pparg^{P465L/+}$

Species	Mutation	Extent of partial lipodystrophy	Insulin resistance	Hypertension
Human	FPLD2 (*LMNA*)	Pronounced fat loss on extremities and gluteal region	Moderate to severe	Moderate to severe
	FPLD3 (*PPARG*)	Moderate fat loss on extremities	Severe	Severe
Mouse	$Pparg^{P465L/+}$	Mild fat redistribution	Minimal or absent	Severe

eficial effects on insulin sensitivity (5). But recent studies of rare patients with loss-of-function mutations in *PPARG* and of mice in which *Pparg* has been manipulated have shown similarities and discrepancies, which underscore PPARγ's physiological complexity and its pleiotropic effects.

Mutations in PPARγ cause human lipodystrophy

In humans, the relationship between PPARγ activity and insulin sensitivity appears to be relatively straightforward: increased PPARγ activity via activating ligands leads to increased insulin sensitivity (5), while reduced receptor activity via germline loss-of-function mutations, such as P467L, leads to insulin resistance (6). Human *PPARG* mutations are associated with the recently identified syndrome familial partial lipodystrophy type 3 (FPLD3, OMIM 604367), which is characterized by relative depletion of subcutaneous fat on extremities along with preservation of central and visceral fat stores. The experiments by Tsai, Maeda, et al. reported in this issue of the *JCI* (7) show that $Pparg^{P465L/+}$ mice — whose genotype is homologous to that of heterozygous $PPARG^{P467L}$ patients — also have repartitioning of adipose stores, albeit in a somewhat different pattern compared with that associated with human FPLD3. These findings, together with previous reports (8–11), firmly establish PPARγ deficiency as a cause of lipodystrophy and confirm the key adipogenic role of PPARγ.

Disproportionate hypertension in $Pparg^{P465L/+}$ mice

The next question is whether lipodystrophy associated with PPARγ deficiency is mechanistically linked with insulin resistance and its complications, particularly hypertension. Among FPLD3 patients with mutant *PPARG*, adipose tissue repartitioning had been proposed to explain, at least partially, insulin resistance and hypertension, largely through analogy

with other lipodystrophies of different molecular etiologies, since insulin resistance is a prominent component of each of these (6). In lipodystrophies, reduced fat storage capacity has been thought to result in increased circulating fatty acids and ectopic triglyceride storage in such sites as skeletal muscle, leading to insulin resistance with consequent development of complications, including hypertension. However, Tsai et al. show that $Pparg^{P465L/+}$ mice did not develop significant insulin resistance (7), in contrast to the severe insulin resistance seen in human $PPARG^{P467L}$ heterozygotes (8). This disparity might be due to a species difference in which human PPARγ retains a more direct link with insulin resistance. Also, the greater relative loss of adipose tissue in human $PPARG^{P467L}$ heterozygotes compared with $Pparg^{P465L/+}$ mice suggests that adipose tissue loss might still contribute to insulin resistance in human PPARγ deficiency.

On closer inspection, however, the extent of lipodystrophy among patients with mutant *PPARG* similarly seems insufficient to account fully for the severity of insulin resistance. In this regard, it is instructive to compare FPLD3 with another autosomal dominant form of partial lipodystrophy, namely FPLD2 (OMIM 151660), which results from mutations in *LMNA* encoding nuclear lamin A/C (6). Like patients with FPLD3, FPLD2 patients have site-specific adipose tissue loss, followed by insulin resistance with hypertension and dyslipidemia, which become even worse as patients age and develop T2DM (12). However, analysis of metabolic subphenotypes indicated that fat loss was more extensive among patients with mutant *LMNA* (FPLD2) than those with mutant *PPARG* (FPLD3) (13). In contrast, insulin resistance and hypertension were more severe among patients with FPLD3 than those with FPLD2 (13). Thus, insulin resistance and hypertension in FPLD3 seemed to be disproportionate

to the extent of lipodystrophy compared with FPLD2, which would be consistent with additional independent effects of mutant *PPARG* (6, 13). The findings in the $Pparg^{P465L/+}$ mice further weaken the case for direct links among adipose redistribution, insulin resistance, and hypertension (Table 1). Could the mutation itself directly mediate hypertension?

PPARγ modulates the renin-angiotensin system

Tsai et al. (7) show that the *PPARG* mutation independently affects other pathways, in particular the renin-angiotensin system (RAS). Both human $PPARG^{P467L}$ heterozygotes and $Pparg^{P465L/+}$ mice are hypertensive, despite the fact that the mice are minimally insulin resistant. The hypertension in the $Pparg^{P465L/+}$ mice is associated with increased expression of RAS components in various adipose depots, specifically angiotensinogen and the angiotensin II (ATII) receptor subtype 1 in inguinal and gonadal fat, respectively (7). This suggests that impaired adipogenesis might locally activate the RAS, with a potential paracrine role for ATII. Alternatively, mutant PPARγ might have other effects on vascular tone. In any event, the findings suggest a more direct role for PPARγ in blood pressure regulation, possibly through linkage with the RAS. Such a link could be one reason why blood pressure decreases with thiazolidinedione treatment (14) and also why hypertensive heterozygotes for the *PPARG* loss-of-function mutation F388L respond well to angiotensin-converting enzyme inhibitors (10).

Thus, Tsai et al. have provided novel insights that advance our understanding of PPARγ physiology (7). In both humans and mice, heterozygous PPARγ mutations are associated with lipodystrophy, but in the mouse there is apparently an uncoupling between the adipose repartitioning and hypertension. Furthermore, the hypertension in the $Pparg^{P465L/+}$ mice might be functionally linked with RAS

activity in adipose tissue. The studies cannot resolve whether human *PPARG* mutations in FPLD3 might act in a dominant negative manner to interfere with function of the normal allele product or whether haploinsufficiency of PPARγ activity is more important. Interestingly, simple haploinsufficiency of PPARγ activity in mice by removal of one *Pparg* allele actually protects against insulin resistance (15), supporting the idea that missense mutations have distinct effects compared with simple reduction in PPARγ. In any event, the findings of Tsai et al. reinforce the importance of PPARγ in adipogenesis (4), highlight the role of adipose tissue as an endocrine organ (16), and also support the idea that *PPARG* mutations affect metabolic and vascular phenotypes through multiple mechanisms, some of which are distinct from effects on adipose tissue mass or distribution.

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1. Laaksonen, D.E., et al. 2002. Metabolic syndrome and development of diabetes mellitus: application and validation of recently suggested definitions of the metabolic syndrome in a prospective cohort study. *Am. J. Epidemiol.* **156**:1070–1077.
2. Lakka, H.M., et al. 2002. The metabolic syndrome and total and cardiovascular disease mortality in middle-aged men. *JAMA.* **288**:2709–2716.
3. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. 2001. Executive summary of the third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III). *JAMA.* **285**:2486–2497.
4. Marx, N., Duez, H., Fruchart, J.C., and Staels, B. 2004. Peroxisome proliferator-activated receptors and atherogenesis: regulators of gene expression in vascular cells. *Circ. Res.* **94**:1168–1178.
5. Stumvoll, M., and Haring, H.U. 2002. Glitazones: clinical effects and molecular mechanisms. *Ann. Med.* **34**:217–224.
6. Hegele, R.A. 2003. Monogenic forms of insulin resistance: apertures that expose the common metabolic syndrome. *Trends Endocrinol. Metab.* **14**:371–377.
7. Tsai, Y.-S., et al. 2004. Hypertension and abnormal fat distribution but not insulin resistance in mice with P465L PPARγ. *J. Clin. Invest.* **114**:240–249. doi:10.1172/JCI200420964.
8. Barroso, I., et al. 1999. Dominant negative mutations in human PPARgamma associated with severe insulin resistance, diabetes mellitus and hypertension. *Nature.* **402**:880–883.
9. Agarwal, A.K., and Garg, A. 2002. A novel heterozygous mutation in peroxisome proliferator-activated receptor-gamma gene in a patient with familial partial lipodystrophy. *J. Clin. Endocrinol. Metab.* **87**:408–411.
10. Hegele, R.A., et al. 2002. *PPARG* F388L, a transactivation-deficient mutant, in familial partial lipodystrophy. *Diabetes.* **51**:3586–3590.
11. Savage, D.B., et al. 2003. Human metabolic syndrome resulting from dominant-negative mutations in the nuclear receptor peroxisome proliferator-activated receptor-gamma. *Diabetes.* **52**:910–917.
12. Hegele, R.A., et al. 2003. Elevated serum C-reactive protein and free fatty acids among nondiabetic carriers of missense mutations in the gene encoding lamin A/C (*LMNA*) with partial lipodystrophy. *Arterioscler. Thromb. Vasc. Biol.* **23**:111–116.
13. Hegele, R.A. 2004. Phenomics, lipodystrophy and the metabolic syndrome. *Trends Cardiovasc. Med.* **14**:133–137.
14. Dobrian, A.D., Schriver, S.D., Khraibi, A.A., and Prewitt, R.L. 2004. Pioglitazone prevents hypertension and reduces oxidative stress in diet-induced obesity. *Hypertension.* **43**:48–56.
15. Miles, P.D., Barak, Y., He, W., Evans, R.M., and Olefsky, J.M. 2000. Improved insulin-sensitivity in mice heterozygous for PPAR-gamma deficiency. *J. Clin. Invest.* **105**:287–292.
16. Engeli, S., et al. 2003. The adipose-tissue renin-angiotensin-aldosterone system: role in the metabolic syndrome? *Int. J. Biochem. Cell Biol.* **35**:807–825.

Innate immunity dictates cytokine polarization relevant to the development of pulmonary fibrosis

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New data support the importance of the innate immune response in the resolution or progression of pulmonary fibrosis. The presence of CXC chemokine receptor 3–expressing cells, specifically pulmonary NK cells, is necessary to produce IFN-γ. This is critical in the polarization of the immune response to injury toward a favorable Th1 response and resolution. In contrast, a Th2 response is associated with progressive fibrosis (see the related article beginning on page 291).

Pulmonary fibrosis is a host response to a variety of known and idiopathic processes and is clinically characterized by insidious onset of dyspnea and abnormal lung function. The pathology of pulmonary fibrosis demonstrates features of dysregulated and abnormal repair with exaggerated vascular remodeling, fibroproliferation, and deposition of extracellular matrix, all leading to progressive fibrosis and loss of lung function. However, the most devastating form is idiopathic pulmonary fibrosis (IPF). IPF is a chronic and often fatal pulmonary disorder with a mortality rate as high as 70% five years after diagnosis (1, 2). The prevalence of IPF increases with age (2, 3). There has been an increase in the incidence of this disorder over the last 10–15 years in many Western countries, including the United Kingdom, New Zealand, and Germany (3). The inability of current immunosuppressive therapies to alter the prognosis of this disorder has renewed scientific interest in multiple areas and has raised additional questions as follows: (a) why are certain individuals susceptible to this disease? do genetic, environmental, or a combination of these factors contribute to the pathogenesis of this disorder? (b) why is the host response to injury to the alveolar capillary wall in susceptible individuals predominated by fibrosis and loss of alveolar architecture instead of resolution? and (c) what are the initial events within the host in response to injury that ultimately set the stage for polarization of the response toward either resolution or perpetuation of fibrosis?